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# Fine Structure of the Vomeronasal Organ in the House Musk Shrew (*Suncus murinus*)

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**ABSTRACT**—We observed fine structures in the vomeronasal organ of the house musk shrew using optical and electron microscopes. The vomeronasal organ of this animal had a pair of openings behind the incisors, and was composed of a pair of blind tubes extending from these. The vomeronasal epithelium was situated on the medial and basal parts in the vomeronasal organ. The medial vomeronasal epithelium was thin and had few basal cells. The basal vomeronasal epithelium was thick and had long sensory cells and numerous basal cells. The distal ends of the vomeronasal sensory cells protruded somewhat into the lumen and had many long microvilli. Further, there were plenty of mitochondria in the cytoplasm. There were only a few secretory granules and mitochondria in the supporting cells. Most of the vomeronasal organ was surrounded by a hard bone capsule, but there was a small window on the postero-dorsal hard bone capsule. On the lateral side, there was a thick sinus along the vomeronasal cavity.

## INTRODUCTION

All vertebrates except for birds and fish have a vomeronasal (Jacobson's) organ which exists on the bottom and/or the under half of the nasal septum. For a long time, this organ has been thought of as a secondary olfactory organ. However, recent studies have shown that the vomeronasal receptors of garter snakes respond well to the odor of food well [3] and that the organ plays a role in the mating behavior of hamsters [6]. Also the vomeronasal organ is well developed anatomically in turtles [4]. The adequate stimulus is, however, still unknown. The mammalian vomeronasal organ is situated in the bottom of the nasal septum, and most of the organ is enclosed by cartilage or hard bone. Carnivora have openings in the oral cavities, and rodents in the nasal cavities [2]. In this study the vomeronasal organ of the house musk shrew, classified as insectivora, was observed using optical and electron microscopes.

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It has been a relatively short time since the house musk shrew was domesticated. This animal belongs to insectivora, which are found not only in tropic and subtropic zones, but also in temperate zone. This animal has induced ovulation by copulation without an estrus cycle [1], and has an interesting, unique mating behavior patterns. When a male and a female animal are put in an aquarium, at first the male encounters the female. After sniffing each other, the female begin to walk at an even pace. The receptive female wags its tail. The male follows the female. The male mounts the walking female. After several mounts, the male succeed in the final intromission and ejaculation. Then the male suddenly change its attitude and begin the postejaculation attack.

## MATERIALS AND METHODS

House musk shrews used in this study were born and raised in our colony at the University of Tsukuba. The origin of this colony was provided in 1987 by Dr. Kitoh (University of Nagoya) and descended from animals trapped in Nagasaki pre-

fecture in the early 1970s. The colony is maintained on a light cycle of 12L : 12D (lights on at 0700) at a temperature of $22 \pm 1^{\circ}C$. They are provided with food (Nippai Trout Food 5P) and water at will. The animals in this study were individually housed (cage dimensions: $30 \times 20 \times 11$ cm) after weaning (21 days of age) with pinewood shavings and paper towels for bedding [7].

The animals were anesthetized with pentobarbital sodium (20–30 mg/kg). The target tissue was fixed through the blood circulation system from the carotid artery by perfusion of the fixative. The fixative is a mixture of 10% formalin, 1% glutaraldehyde, 1% succharose, and 0.1M-Phosphate buffer (pH 7.4). 2% $OsO_4$ was used as a post for electron microscopy. Specimen was decalcified with a 10% EDTA-2Na solution for 4 weeks at 4°C, and were embedded in epoxy resin using the ordinary procedures. Specimens were sectioned into sections 10 $\mu$m thick and stained by toluidin blue for optical microscopy. The sections for electron microscopy were double-stained by uranyl acetate and lead citerate.

## RESULTS AND DISCUSSION

### *Optical microscopy*

Morphological charasteristics of the house musk shrew were recorded in this study. A third of the

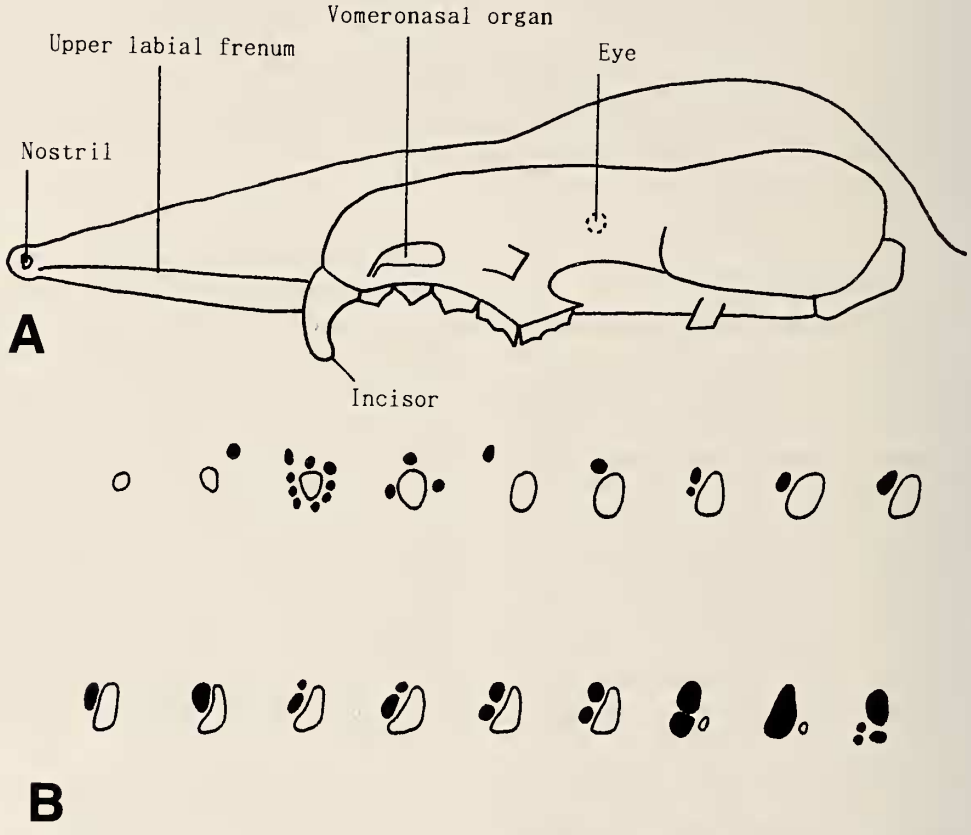


Fig. 1. A The schema of the head of the house musk shrew without the lower jaw. The area of oblique lines shows the location of the vomeronasal organ.
B The schema shows the right halves of the transverse sections at every 150 $\mu$m of the vomeronasal organ. Filled circles show blood vessels. Thick blood vessels are especially called sinuses. Open circles show vomeronasal cavities called lumens. The sinuses were situated on the lateral side of the lumen. The whole length of the vomeronasal cavity was 2250 $\mu$m. The highest length of it was 490 $\mu$m.

whole length of the head was snout. External nares opened on right and left sides of the snout tip (Fig. 1A). The upper labial frenum extended back to the upper lip cleft and reached the openings of the vomeronasal organ through the upper incisors. The length of the duct from the opening to the vomeronasal organ was over 1200 $\mu$m, and 3 $\mu$m in the short diameter and 230 $\mu$m in the long diameter. A house musk shrew always moves its snout up and down rapidly. Labial frenum reached from the tip of the snout to the openings of the vomeronasal organ so that the rapid up and down movement of the snout would be involved in the cominng and going of the stimulant. In rats [9] and hamsters [8], there were a pair of external nares in the blind tube-like vomeronasal organ in the nasal cavity. The length of the blind tube where the vomeronasal epithelium was distributed was 2550 $\mu$m, and 490 $\mu$m at its highest height. The duct to the vomeronasal cavity was enclosed by cartilage, and the anterior third of the vomeronasal cavity was enclosed by hard bone (Fig. 2A). The dorsolateral part of the remaining two thirds was adjacent to the olfactory cavity bordered by connective tissue(Fig. 2B, C). The vomeronasal organ was situated in the bottom of the nasal septum and connected to the palatine. There were some blood vessels along an anterior third of the vomeronasal cavity and a thick sinus on the lateral side along the middle third of it, and the lumen of it was triangular or crescent (Fig. 1B). There were two thick sinuses on the lateral side along the posterior third of it, and the lumen suddenly became small (Fig. 1B, Fig. 2C). At the posterior end of the vomeronasal organ, sinuses gathered into one vessel again, making this point the thickest (Fig. 1B). The vomeronasal epithelium was found only on the medial side of the anterior and middle thirds of the vomeronasal organ (Fig. 2A, B). On the middle third of the vomeronasal organ, the epithelium was found in the dorsal, medial and ventral parts (Fig. 2B). In house musk shrews, the sinus along the lateral side in the vomeronasal organ was not as well-developed as in rats [9] and hamsters [8]. On the posterior third of the vomeronasal organ, it was found in the medial and ventral parts. The thickness of the vomeronasal epithelium was thicker on the dorsal and ventral parts than that on the medial part (Fig. 2). The blind tube-like vomeronasal cavity was filled with mucus and this mucus should carry stimulant into the cavity. It is thought that the coming and going of stimulant in the cavity is controlled by the pumping of the sinous, which is controlled of the sympathetic nerve [5].

*Electron microscopy*

There were three types of cells in the vomeronasal epithelium: vomeronasal cells, supporting cells and basal cells (Fig. 3A). The vomeronasal cell was a bipoler cell, and the distal end protruded into the lumen. The protrusion had more than 100 microvilli. The length of each microvilus was more than 10 $\mu$m, with a diameter about 0.1 $\mu$m (Fig. 3B). The distal end had numerous mitochondria, especially in the protrusion there were some centrioles (Fig. 3B). There were no cilia. The dendrite of an olfactory cell had tonofilaments, but we could not observe tonofilaments and microtubles in the vomeronasal cell. The ovoidal nuclei were in a line on the middle layer or the basal layer within the epithelium. Mitochondria, Golgi body, and free ribosomes were observed around the perinuclear area. We could not observe smooth endoplasmic reticulum. Supporting cells had cylindrical nuclei in the middle layer within the epithelium (Fig. 3A). There were short processes on the distal ends of the supporting cells (Fig. 3A). Mitochondria and rough endoplasmic reticula lied scattered over the cytoplasm and there was a Golgi body in the perinuclear area. The cytoplasm and the nucleus of the supporting cell had lower electron densities than those of the vomeronasal cell. The supporting cell had no secretory granules. The basal cells were situated on the basal layer of the epithelium, and the cells and the nuclei were flat and somewhat horizontal (Fig. 3A). The nucleus occupied most of the cell. We could not find tonofilaments. The basal cells gathered in the thick part of the epithelium, the dorsal part and the ventral part of the vomeronasal epithelium. No one has studied whether the basal cells are the stem cells of the vomeronasal cells, but it seems that they are the stem cells of the vomeronasal cells from the results of the developmental study [8]. We could not find tonofilaments on both of

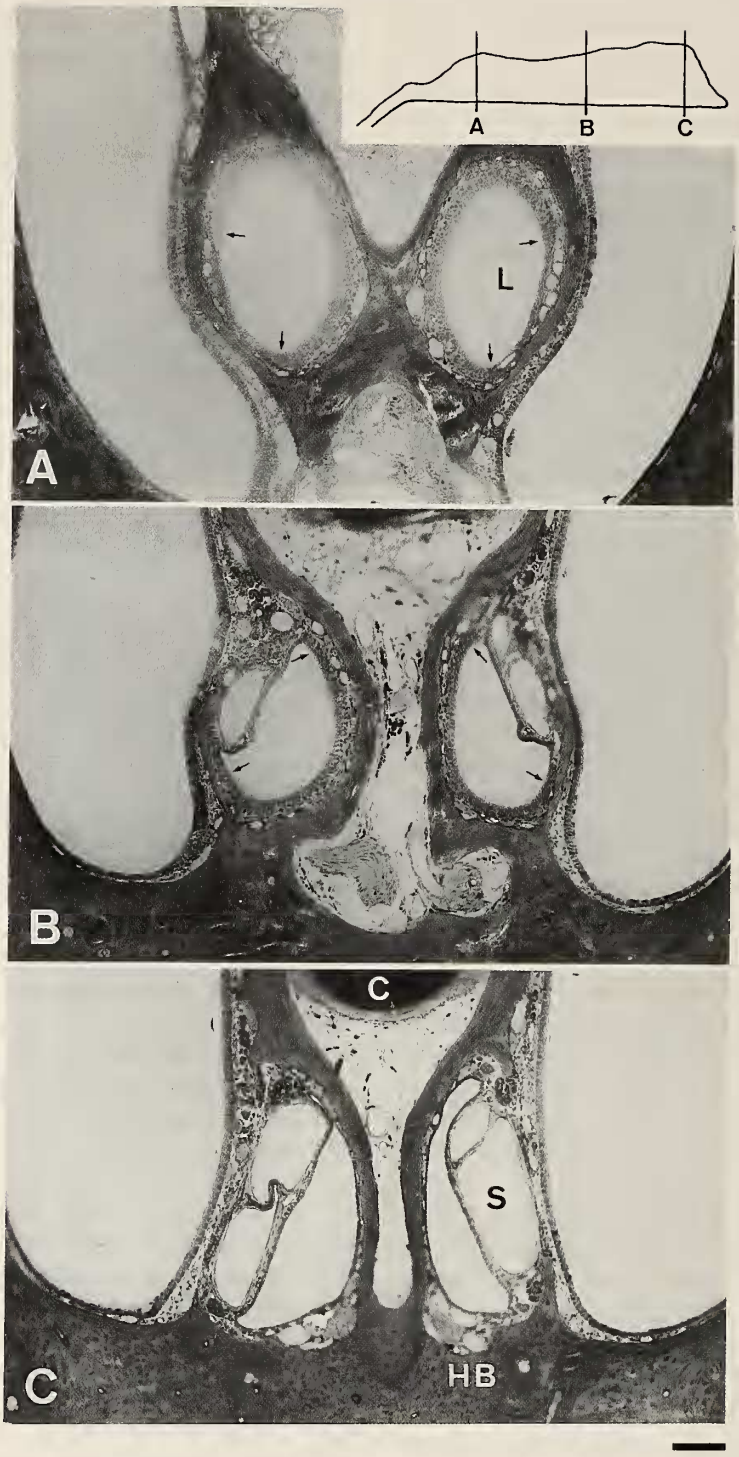


Fig. 2. The transverse sections of the vomeronasal organ. Scale 100 $\mu$m. The inlet shows the locations of the section A, B and C in the vomeronasal organ. arrows: bordres between vomeronasal mucosa and stratified layers. C cartilage. HB hard bone. L lumen. A an anterior third. B a middle third. C a posterior third.

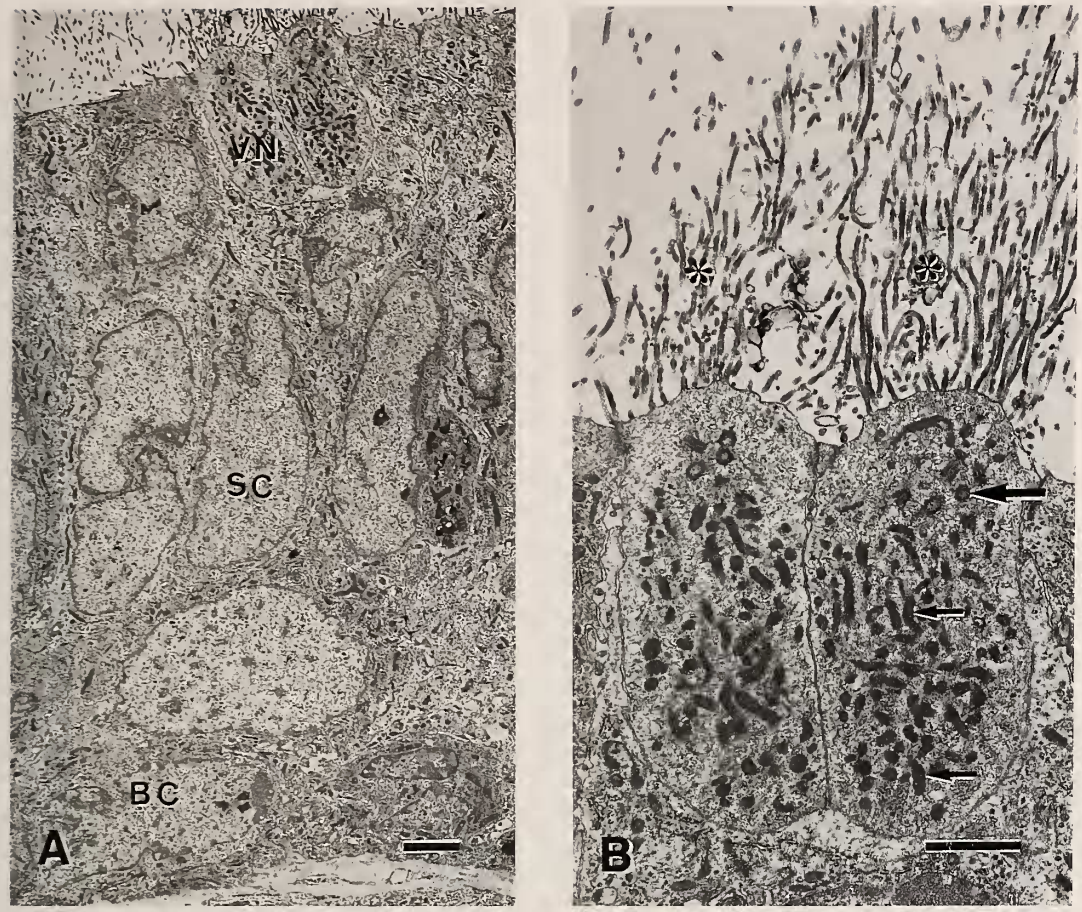


FIG. 3. Electron micrographs of the vomeronasal epithelium. asterisks: microvilli. Small arrows mitochondria. Large arrow a centriole. BC a basal cell. SC a supporting cell. VN a vomeronasal receptor cell.
A A picture of the whole height of the vomeronasal epithelim. Scale 2 $\mu$m. B Distal ends of the vomeronasal receptor cells. Scale 5 $\mu$m.

supporting and vomeronasal cells. Cell arrangement among the three kinds of cells was not as regular as that in the olfactory epithelium. The surface of the lateral vomeronasal organ was a stratified layer with short processes. In rats, mice and rabbits, They have vomeronasal respiratory epithelium with cilia [10], but house musk shrews did not have it.

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